THE SYNTHESIS OF 3-AMINO-3-DEOXY-D-GLUCOSE (KANOSAMINE) AND ITS 1,6-ANHYDRO DERIVATIVE CONFORMATION OF AMINO DERIVATIVES OF 1,6-ANHYDRO-β-D-HEXOPYRANOSES*

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3-Amino-1,6-anhydro-3-deoxy-β-D-glucopyranose (*VI*) was prepared in 85% yield by ammonolysis of a mixture of 1,6 : 3,4- (*IV*) and 1,6 : 2,3-dianhydro-β-D-allopyranose (*V*), formed from 1,6-anhydro-2,4-di-O-benzyloxycarbonyl-3-O-methanesulfonyl-β-D-glucopyranose (*III*) on reaction with sodium methoxide in chloroform. On hydrolysis of amino derivative *VI* with hydrochloric acid and deionization of the hydrolytic product crystalline 3-amino-3-deoxy-D-glucose (*IX*) was obtained. On the basis of the interpretation of the ^{1}H-NMR spectra of some 1,6-anhydro-β-D-hexopyranoses and their amino derivatives it was found that both the amino derivative *VI* and its hydrochloride *VII* occur mainly in the boat conformation and not in the usual chair conformation.

Kanosamine, 3-amino-3-deoxy-D-glucose, is a component of the antibiotics kanamycin[1], nebramycin[2] and hikizimycin[3]. Since its structure was elucidated[4] the synthesis of kanosamine and its derivatives has been investigated by several authors[5-10]; it may be also prepared by fermentation of *Bacillus aminoglucosidicus*[11]. The majority of the mentioned syntheses, among which that starting from 1,2 : 5,6-di-O-isopropylidene-α-D-glucofuranose seems most convenient, makes mainly the N-acetyl derivative of kanosamine accessible[8-10].

In this paper we describe an alternative synthesis of kanosamine (*IX*) and of its 1,6-anhydro derivative *VI*, and we endeavour to explain the effect of the amino group on the conformation of these compounds on the basis of ^{1}H-NMR spectra of some amino derivatives of 1,6-anhydro-β-D-hexopyranoses. The starting compound is 1,6-anhydro-β-D-glucopyranose (*I*) which is converted on reaction with benzyloxycarbonyl chloride in pyridine[12] to 2,4-di-O-benzyloxycarbonyl derivative *II*, and then by subsequent mesylation[13] to 3-O-methanesulfonyl ester *III*. When reacting the

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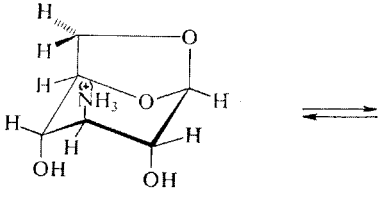
compound *III* with sodium methoxide in chloroform a mixture containing 1,6 : 3,4--dianhydro-β-D-allopyranose (*IV*) and 1,6 : 2,3-dianhydro-β-D-allopyranose (*V*) was formed from it in an approximately 3 : 1 ratio. On reaction of ethanolic ammonia with a mixture of dianhydro derivatives *IV* and *V* (an analogous reaction of sodium methoxide see ref.[14]) 3-amino-1,6-anhydro-3-deoxy-β-D-glucopyranose (*VI*) was obtained in 85% yield, which is also formed under the same conditions from pure components of the mixture. During ammonolysis no decomposition was observed, which usually takes place during the heating of dianhydro derivatives *IV* and *V* in a 5% potassium hydroxide solution[15].

The structure of 1,6-anhydrokanosamine *VI* has been demonstrated by converting it to the known hydrochloride[16] *VII* and also to 3-acetamido-2,4-di-O-acetyl-1,6--anhydro-3-deoxy-β-D-glucopyranose (*VIII*) and comparing the physical constants and the IR spectra of the latter compound with those of an authentic sample. Crystalline 3-amino-3-deoxy-D-glucose (*IX*, kanosamine) was prepared on hydrolysis of 1,6-anhydrokanosamine *VI* with approximately 20% hydrochloric acid and deionization of the hydrochloride formed.

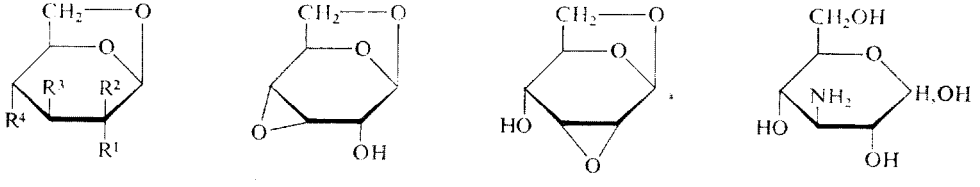
We also tried to confirm the chemically demonstrated structure of 1,6-anhydrokanosamine *VI* by ^{1}H-NMR measurements. As the results did not correspond to the expected $^1C_4$ conformation of substance *VI*, we also compared the ^{1}H-NMR spectra of other amino derivatives of 1,6-anhydro-β-D-hexopyranoses, their hydrochlorides and two corresponding model substances (Table I). The presence of the ammonium group in the spectrum was indicated by the upfield shift of the C—H proton in the position of the substitution. Its signal is always located at the highest field. Hydrochlorides of 2-amino and 4-amino derivatives *X* or *XI*, resp., with a *gluco* configuration, generally display low values of vicinal coupling constants of hydrogens H-1 to H-5 (approximately 1 to 2 Hz), in agreement with their equatorial positions in the supposed chair conformation $^1C_4$ of the pyranose cycle. The relatively high ^4J long-range couplings $J_{1,3}$, $J_{2,4}$ and $J_{3,5}$ observed, characteristic of equatorial protons in an approximately flat zig-zag arrangement, also correspond to this conformation. Similar values for coupling constants (higher by tenths of Hz only) were also obtained under the same conditions for levoglucosan *I* in which a hydroxyl group stands instead of an amino group. The hydrochloride of 2-amino derivative *XIII* with *manno*-configuration also afforded the expected low values for vicinal coupling constants, and similar data as *manno* derivative *XII*. The increased $J_{2,3}$ value and the disappearance of the long-range coupling $J_{2,4}$ (in contrast to *X* and *XI*) are evident consequences of the changed configuration of $C_{(2)}$ where the proton H-2 assumes an axial position with simultaneous preservation of the chair $^1C_4$ conformation of the pyranose cycle.

In the case of the hydrochloride of 3-amino derivative *VII* high coupling constant values were found, $J_{2,3}$ and $J_{3,4}$ (about 8 Hz), and extremely low $J_{1,2}$ (about 0 Hz) which differ considerably from the coupling constants of the preceding two amino

hydrochlorides with *gluco* configuration (*X* and *XI*) and of levoglucosan *I*. The observed changes cannnot be elicited by the inductive effect of the substituents on the magnitude of *J* only (the differences in empirical electronegativities[17] of the groups OH (3·7), NH_2 (3·35), and NH_3^+ (3·8) are small), which, by estimation, should not exceed several tenths of Hz. The observed $J_{2,3}$ and $J_{3,4}$ values necessarily indicate a *trans*-axial arrangement of the protons in the positions 2,3 and 3,4, which may be expected, for example, in $^1C_4$ conformation of 1,6-anhydro derivative with an *ido* configuration. The formation of 3-amino-1,6-anhydro-3-deoxy-β-D-idopyranose[18] by ammonolysis of dianhydro derivatives *IV* and *V* is excluded, however. The coupling constants values must therefore be a consequence of the change in conformation. From an analysis of models it follows that the pyranose ring in derivative *VII* must assume the boat $B_{0,3}$ conformation in which the dihedral angles of the protons 2,3 or 3,4 are approximately 170° and of the protons 1,2 about 100°, *i.e.* in agreement with the magnitudes of the corresponding coupling constants. In order to be able to esti-



VIIa *VIIb*



IV *V* *IX*

I, $R^1, R^3, R^4 = OH$, $R^2 = H$
II, $R^1, R^4 = C_6H_5CH_2OCOO$, $R^2 = H$, $R^3 = OH$
III, $R^1, R^4 = C_6H_5CH_2OCOO$, $R^2 = H$, $R^3 = CH_3SO_2O$
VI, $R^1, R^4 = OH$, $R^2 = H$, $R^3 = NH_2$
VII, $R^1, R^4 = OH$, $R^2 = H$, $R^3 = NH_3Cl$
VIII, $R^1, R^4 = CH_3COO$, $R^2 = H$, $R^3 = CH_3CONH$
X, $R^1 = NH_3Cl$, $R^2 = H$, $R^3, R^4 = OH$
XI, $R^1, R^3 = OH$, $R^2 = H$, $R^4 = NH_3Cl$
XII, $R^1 = H$, $R^2, R^3, R^4 = OH$
XIII, $R^1 = H$, $R^2 = NH_3Cl$, $R^3, R^4 = OH$

mate the effect of the positive charge on nitrogen in amine hydrochloride *VII* we also measured its spectrum in alkaline aqueous solution (30% NaOD in D_2O) in which only the amino form *VI* should be present. Under these conditions the observed values of $J_{2,3}$ and $J_{3,4}$ decreased to 5·5–6·0 Hz. Approximately equal coupling constants $J_{2,3}$ and $J_{3,4}$ (approx. 5·5 Hz) were also found in the spectrum of 3-amino derivative *VI* itself in hexadeuteriodimethyl sulfoxide. After acidification of the dimethyl sulfoxide solution of amino derivative *VI* with a small amount of CD_3COOD not only the signals of exchangeable protons disappeared, but the coupling constants $J_{2,3}$ and $J_{3,4}$ also changed (increasing to 8·1 Hz) as did chemical shifts of other protons to values very close to those found for amine hydrochloride *VII* in hexadeuteriodimethyl sulfoxide. The changes observed can be interpreted by the shift of the dynamic equilibrium of the chair and the boat forms *VIIa* and *VIIb* (fast from the point of view of the ^{1}H-NMR time scale) practically completely to the boat form *VIIb* side under the effect of the protonation of the 3-amino group, while, evidently, the nature of the anion of the ammonium salt formed does not matter too much. On using the values approximately 2·5 Hz for the $J_{2,3}$ or $J_{3,4}$ values for the chair form (taken over from the values of levoglucosan *I*) and approx. 8·0 Hz for the boat form (from *VII*) the equilibrium ratio of the chair-boat conformers may be estimated as 45 : 55 for amino derivative *VI* in hexadeuteriodimethyl sulfoxide solution. The observed upfield shift of the H-6_{endo} proton, caused evidently by the elimination of the van der Waals deshielding effect of the $C_{(3)}$-substituent during the transition to the boat conformation is also consistent with the shift of the equilibrium position in the direction of the boat form during the protonation of the amino group.

The reasons leading to the transition of the pyranose cycle from the chair form to the boat form when the hydroxyl group is substituted by an amino group, and further when the amino group is protonated, might, in our view, be the following: the stability of the levoglucosan *I* form with axial substituents in the positions 2, 3 and 4 is undoubtedly enhanced by the antiparallel orientation of the dipoles of the C—OH bonds with maximum possible distance of the hydroxyl oxygens. In the boat form the dipoles of the equatorial bonds C—OH should assume an approximately parallel arrangement with electrostatic repulsion of mutually close oxygen atoms. In the case of hydrochloride *VII* the orientation of the $C_{(3)}$-substituent-bond dipole is changed and the boat form with a parallel arrangement of the oppositely polarized equatorial bonds becomes more advantageous from the point of view of electrostatic interactions. Further important factors which might take part in the conformational preference are the intramolecular hydrogen bonds and interactions with the solvent (solvation). The special arrangement of levoglucosan *I* in the chair form permits, theoretically, all hydroxyl protons to be engaged in intramolecular hydrogen bonds, while the atoms of oxygen $O_{(5)}$ and $O_{(6)}$ may function as electron donors, as was confirmed by earlier measurements of the IR spectra of model substances from the

TABLE I

Characteristic Parameters of ^{1}H-NMR Spectra

Chemical shifts and coupling constants were obtained by first order analysis. The coupling constants indicated with ~ were read with an accuracy less exact than 0·5 Hz. Unless stated otherwise the solvent was hexadeuteriodimethyl sulfoxide, and hexamethyldisiloxane served as reference (chemical shifts are corrected for tetramethylsilane according to the relation $\delta_{HMDS} = 0{\cdot}06$ p.p.m.).

Compound	Chemical shifts						
	H-1	H-2	H-3	H-4	H-5	H-6en	H-6ex
I	5·19	3·22	3·44	3·35	4·38	3·94	3·54
I[a]	5·36	3·43	3·59	3·59	4·55	4·00	3·65
VI	5·15	3·05	2·55	3·20	4·32	3·78	3·50
VI[b]	5·18	3·37	2·66	3·53	4·38	3·68	3·55
VII	5·17	3·46	2·66	3·63	4·41	3·69	3·61
X	5·57	2·99	3·70	3·54	4·54	4·14	3·63
XI	5·31	3·38	3·66	3·13	4·70	4·18	3·65
XII[a]	5·31	3·68	3·86	3·85	4·51	4·14	3·65
XIII	5·48	3·24	3·85	3·67	4·47	4·19	3·60

Compound	Coupling constants									
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6en}$	$J_{5,6ex}$	$J_{6,6}$	$J_{1,3}$	$J_{2,4}$	$J_{3,5}$
I	1·2	2·6	2·4	2·3	1·1	5·9	7·1	1·4	1·1	1·2
I[a]	1·7	~2·0	[d]	2·2	1·0	5·8	7·5	1·3	1·3	[d]
VI	~0	~5·5	~5·5	~1·0	~1·0	~5·0	~7·5	~0·5	~0·5	~0·5
VI[b]	~0	8·1	8·1	~1·0	0·9	4·7	7·4	~0	~0	~0
VII	~0	~8·0	~8·0	~1·0	~1·5	~4·5	~7·5	~0	~0	~0
VII[c]	~0	~6·0	~5·5	~1·0	[d]	[d]	[d]	~0·5	~0·5	~0·5
X	~1·0	~1·0	~2·0	~1·0	~1·0	~6·0	~7·5	~1·0	~1·0	~1·0
XI	1·3	1·3	1·5	~1·0	0·7	5·7	7·3	1·3	1·0	[d]
XII	1·7	[d]	[d]	1·8	1·2	6·0	7·5	1·3	~0·5	[d]
XIII	1·8	5·8	1·5	~1·5	0·8	6·0	7·0	1·2	~0·5	1·5

[a] The data were taken from literature[24]; in deuterium oxide with sodium 2,2-dimethyl-2-silapentane-5-sulfonate as standard; [b] CD_3COOD added; [c] in a 30% solution of NaOD in D_2O; [d] *J* values could not be determined.

series of 1,6-anhydro-β-D-hexopyranoses[19,20]. The question to what extent the formation of these intramolecular hydrogen bonds would be impaired in the polar solvents used (hexadeuteriodimethyl sulfoxide, D_2O) by intermolecular associations with the solvent can be answered unambiguously only with difficulty, and it will evidently depend on the strength of the hydrogen bonds. In the boat form of levoglucosan *I* the possibility of the formation of intramolecular hydrogen bonds between the hydroxyl protons and oxygen atoms $O_{(5)}$ and $O_{(6)}$ disappears. The substitution of the hydroxyl group on $C_{(3)}$ by the amino group or ammonium group (*VI* or *VII*) may lead to a weakening or even the disappearance of the hydrogen bond with $O_{(6)}$ of the anhydro bridge and to an increase in solvation of the $C_{(3)}$-substituent. A steep increase of the effective volume of axial $C_{(3)}$-substituent can then contribute to the destabilization of the chair conformation in consequence of steric interactions.

EXPERIMENTAL

The melting points were measured on a Boëtius micromelting point apparatus, and the optical rotations on an automatic polarimeter of Bendix-Ericsson, type 143 A, at 23–25°C. The ^{1}H-NMR spectra were measured on a Varian HA-100 instrument (at 100 MHz). The purity of amino derivatives was controlled on an automatic Amino Acid Analyzer AAA 881 of Microtechna, Prague, in citrate buffers of pH 5·28 and 3·25. The solutions were concentrated under reduced pressure at temperatures below 50°C. Samples for analysis were dried over phosphorus pentoxide at 0·2 Torr.

Hydrochlorides of 2-amino-1,6-anhydro-2-deoxy-β-D-glucopyranose[21] (*X*) and 2-amino-1,6-anhydro-2-deoxy-β-D-mannopyranose[16] (*XIII*) were prepared according to literature. The as yet undescribed hydrochloride of 4-amino-1,6-anhydro-4-deoxy-β-D-glucopyranose[22] (*XI*) had m.p. 160–180°C (decomp.), $[\alpha]_D$ −65° (*c* 0·82, water).

1,6 : 3,4-Dianhydro-β-D-allopyranose (*IV*) and 1,6 : 2,3-Dianhydro-β-D-allopyranose (*V*)

Methanol (100 ml) in which sodium (8 g) was dissolved was added dropwise and under stirring and cooling (approx. 5°C) to a solution of 20 g of 1,6-anhydro-2,4-di-O-benzyloxycarbonyl-3-O-methanesulfonyl-β-D-glucopyranose[13] (*III*) in 70 ml of chloroform. The mixture was allowed to stand at room temperature for 15 hours and then neutralized with hydrochloric acid. The neutral solution was evaporated by distillation and the residue extracted, while still warm, with five 100 ml portions of acetone. After distilling off of acetone the residue was dissolved in water and deionized on a column of Bio-Deminrolite and the solution was evaporated again. After drying the residue over phosphorus pentoxide 4·25 g (76%) of a syrupy mixture was obtained which contained dianhydro derivatives *IV* (76%) and *V* (24%), as determined by gas chromatography: glass column 190 × 0·3 cm, Gas Chrom Q (80–100 mesh), with 10% SP 2401, T_c 124°C, T_i 155°C, nitrogen flow 22 ml/min; dianhydro derivative *IV*, t_r = 5·0 min, dianhydro derivative *V*, t_r = 6·5 min.

3-Amino-1,6-anhydro-3-deoxy-β-D-glucopyranose (*VI*)

A solution of dianhydro derivative *IV* and *V* (4 g) in 80 ml of a 14% ethanolic ammonia was heated in a stainless steel pressure vessel at 120–125°C for 50 hours. After evaporation of the

reaction mixture the residue was crystallized from ethanol. Yield, 3·8 g (85%) of substance *VI*, m.p. 134–135°C, $[\alpha]_D$ −57° (*c* 0·7, water). For $C_6H_{11}NO_4$ (161·2) calculated: 44·71% C, 6·88% H, 8·69% N; found: 44·59% C, 6·95% H, 8·53% N.

Hydrochloride hydrate of VII: m.p. 70–84°C, $[\alpha]_D$ −50° (*c* 0·9, water) in agreement with the literature[16].

Triacetyl derivative VIII: This was prepared by acetylation of *VI* with acetic anhydride in pyridine, m.p. 177–178°C, $[\alpha]_D$ −65·5° (*c* 0·84, chloroform). The physical constants and the IR spectra are in agreement with those of an authentic specimen[16].

3-Amino-3-deoxy-D-glucose (*IX*)

A solution of 100 mg of 1,6-anhydro derivative *VI* in 4 ml of azeotropic hydrochloric acid (approximately 6M-HCl) was refluxed under an inert gas for 20 minutes. The reaction mixture was concentrated, the residue dissolved in 10 ml of water decolorized with charcoal and the solution was deionized on a column of Amberlite IR 400 in OH^- cycle. After evaporation of water the residue was crystallized from methanol. The yield of kanosamine *IX* was 77 mg (72%), m.p. 130–135°C (under decomposition), $[\alpha]_D$ +35° (5 min) → +50° (7 hours, const.: *c* 0·76, water). Literature[1] gives m.p. 131·5–137°C (decomp.), $[\alpha]_D^{21}$ +50·5° (*c* 1·0, water) without mentioning mutarotation, literature[11] gives m.p. 140–143°C (decomp.), $[\alpha]_D^{16}$ + 39 → +19° (24 hours, *c* 0·9, water), literature[23] gives $[\alpha]_D^{14}$ +19° (1 to 4 hours, *c* 1·0, water). Paper chromatography: paper Whatman No 1, solvent system 1-butanol–acetic acid–water (4 : 2 : 1), detection with 0·5% ethanolic ninhydrin, R_F 0·16 (kanosamine *IX* traces of a substance with a lower R_F value also present, R_F 0·124 (D-glucosamine hydrochloride).

Hydrochloride of kanosamine IX: This was prepared in ethanolic hydrogen chloride (it is hygroscopic), m.p. about 150°C (decomp.), $[\alpha]$+55° (5 min) → +50·5° (25 min, const.; *c* 1·34, water); literature[6] gives $[\alpha]_D^{20}$ +47° (5 min) → +43° (6 hours, const., *c* 1·0, water), literature[11] m.p. 115–120°C (decomp.), $[\alpha]_D^{20}$ +33° (one hour, *c* 0·91, water), literature[23] $[\alpha]_D^{14}$ +58° (*c* 1·0, water).

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REFERENCES

1. Murase M.: J. Antibiot. *14* (A), 367 (1961).
2. Koch K. F., Rhoades J. A.: Antimicrob. Ag. Chemother. *1970*, 309.
3. Das B. C., Defaye J., Uchida K.: Carbohyd. Res. *22*, 293 (1972).
4. Cron M. J., Evans D. L., Palermiti F. M., Whitehead D. F., Hooper I. R., Chu P., Lemieux R. U.: J. Amer. Chem. Soc. *80*, 4741 (1958).
5. Kuhn R., Baschang G.: Justus Liebig's Ann. Chem. *628*, 206 (1959).
6. Baer H. H.: J. Amer. Chem. Soc. *83*, 1882 (1961).
7. Williams D. T., Jones J. K. N.: Can. J. Chem. *45*, 7 (1967).
8. Brimacombe J. S., Bryan J. G. H., Husain A., Stacey M., Tolley M. S.: Carbohyd. Res. *3*, 318 (1967).
9. Meyer W. zu Reckendorf: Chem. Ber. *101*, 3802 (1968).
10. Ježo I.: Chem. Zvesti *27*, 681 (1973).
11. Umezewa S., Umino K., Shibahara S., Hamada M., Omoto S.: J. Antibiot. *20* (A), 355 (1967).

12. Černý M., Gut V., Pacák J.: This Journal *26*, 2542 (1961).
13. Černý M., Trnka T., Beran P., Pacák J.: This Journal *34*, 3377 (1969).
14. Seib P.: J. Chem. Soc. (C) *1969*, 2552.
15. Trnka T., Černý M.: This Journal *36*, 2216 (1971).
16. Černý M., Elbert T., Pacák J.: This Journal *39*, 1752 (1974).
17. Wells P. R.: Progr. Physical Organic Chem. *6*, 127 (1968).
18. Richardson A. C., Fischer H. O. L.: J. Amer. Chem. Soc. *83*, 1132 (1961).
19. Wollwage P. C., Seib P. A.: J. Chem. Soc. (C) *1971*, 3143.
20. Pecka J., Staněk J. jr, Černý M.: This Journal *39*, 1192 (1974).
21. Černý M., Juláková O., Pacák J.: This Journal *39*, 1391 (1974).
22. Černý M., Černý I., Pacák J., Trnka T.: This Journal, in press.
23. Maeda K.: *Streptomyces Products Inhibiting Mycobacteria*, p. 69. Wiley, New York 1965.
24. Heyns K., Weyer J.: Justus Liebig's Ann. Chem. *718*, 224 (1968).

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